PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
A61K 37/00, 47/48, 37/54, C12N 9/42, 9/96, 11/00

A1

(11) International Publication Number: WO 94/13311

(43) International Publication Date: 23 June 1994 (23.06.94)

(21) International Application Number: PCT/US93/11920

(22) International Filing Date: 8 December 1993 (08.12.93)

(30) Priority Data:
07/989,802 10 December 1992 (10.12.92) US

(71) Applicant: ENZON, INC. [US/US]; 40 Kingsbridge Road, Piscataway, NJ 08854 (US).

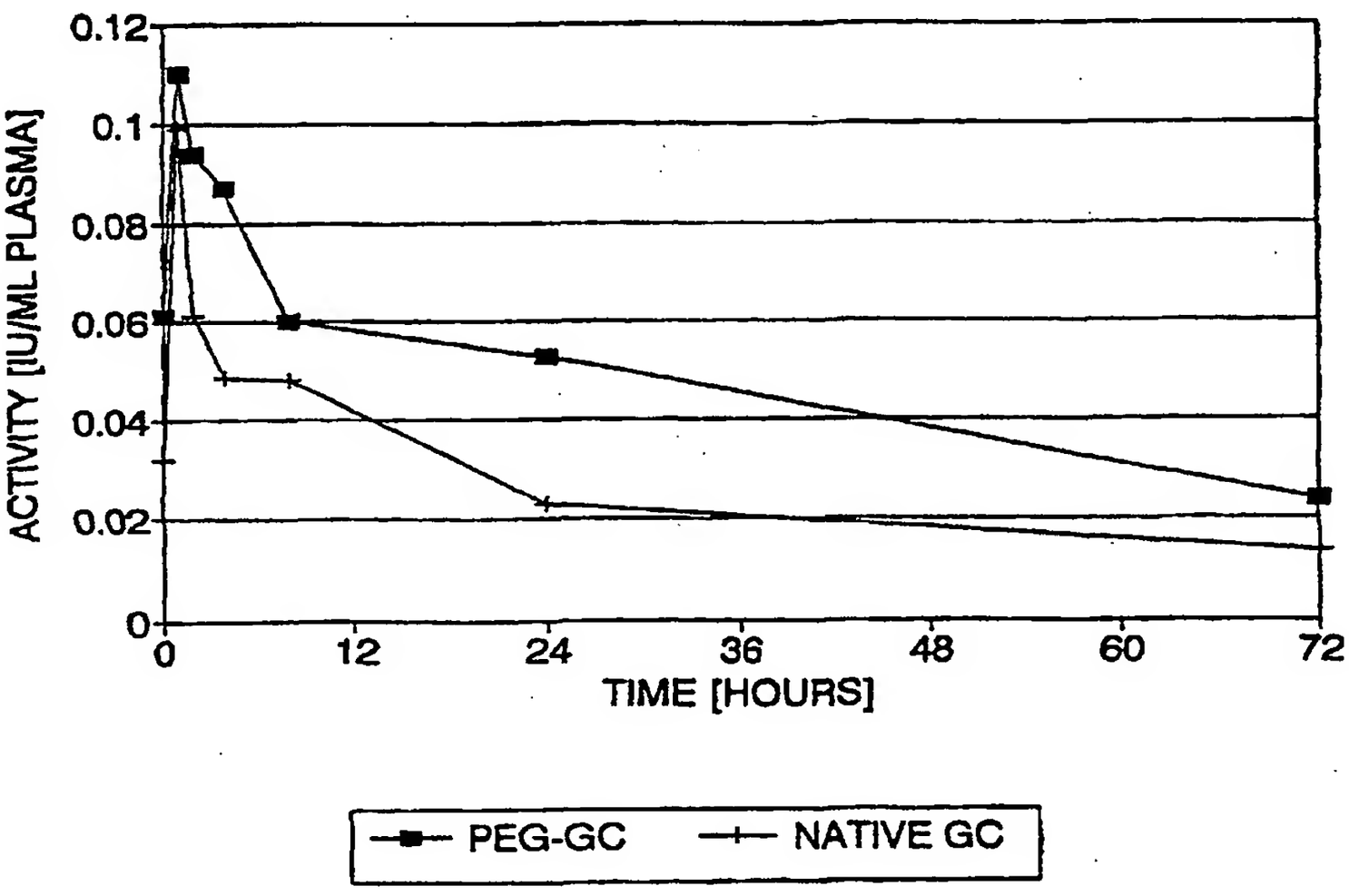
(72) Inventors: SHORR, Robert, G., L.; 28 Brookfall Road, Edison, NJ 08820 (US). CHO, Myung-Ok; 166 A Cedar Lane, Highland Park, NJ 08904 (US). GILBERT, Carl, W.; 26 Hampton Court, Basking Ridge, NJ (US).

(74) Agents: MERCANTI, Michael, N. et al.; Enzon, Inc., 40 Kingsbridge Road, Piscataway, NJ 08854 (US).

(81) Designated States: AU, BG, BR, CA, CZ, FI, HU, JP, KP, KR, NO, NZ, PL, PT, RO, RU, SK, UA, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: GLYCOLIPID ENZYME-POLYMER CONJUGATES



(57) Abstract

Conjugates containing substances having activity against glycolipids such as glucocerebroside and non-antigenic polymers such as polyethylene glycol are disclosed. The conjugates circulate for extended times and have prolonged activity *in vivo* when compared to unmodified enzymes. The conjugates are useful in the treatment of Gaucher's disease.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgyzstan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

GLYCOLIPID ENZYME-POLYMER CONJUGATES

BACKGROUND OF THE INVENTION

The present invention relates to conjugates having prolonged hydrolyzing activity in vivo against glycolipids such as glucocerebroside.

Gaucher's disease is an autosomal recessive genetic disorder which effects about 20,000 people in the Unites States. The disease is the most common lysosomal storage disorder and describes a defect in the afflicted's naturally-occurring glucocerebrosidase (GCS). This defect causes pathological storage of the complex lipid, glucocerebroside, primarily in organs and tissues of the reticuloendothelial system. The disease is systemic and patients may experience enlargement of the liver and spleen as well as replacement of the bone marrow with lipid-filled cells known as Gaucher cells.

Unfortunately, there is currently no cure for patients suffering from this disease. Treatment for the disease is largely symptomatic. For example, analgesics are used for relief of pain, blood and platelet transfusions are often indicated. In cases where the disease is severe, a splenectomy is indicated to remove the enlarged spleen.

Gaucher's disease is considered to be a good candidate for enzyme-replacement therapy. For example, U.S. Patent No. 3,910,822 discloses the use of GCS isolated from human placental tissue as a treatment of Gaucher's disease. In addition, PCT Publication Nos. WO 90/07573 and WO 89/05850 describe preparing GCS using recombinant DNA techniques. While these advancements are significant, effective treatment and management of the

disease for many patients remains elusive. A chief drawback with current therapies is the relatively short period of time that the replacement enzyme is active in vivo. Accumulated glucocerebroside is not always fully metabolized. Thus, the long term effects of the lingering glycoprotein has not been addressed.

U.S. Patent No. 4,935,465 describes protein conjugates including glucocerebrosidase reversibly linked to water soluble polymers. The linking groups described therein are based on maleic acid derivatives which are quickly hydrolyzed in vivo and thus release the unmodified protein from the polymer. The present inventors, however, have found that alternative linking groups having a much greater resistance to in vivo hydrolysis provide conjugates which are enzymatically-active over longer periods and thus are better suited to act on accumulated glycolipids and thus reduce the residual amounts of glucocerebroside in the plasma and normalize spleen, liver and skeletal abnormalities.

SUMMARY OF THE INVENTION

The present invention provides biologically active conjugates having prolonged activity against glycolipids such as glucocerebroside. The conjugates contain a substance such as an enzyme or enzyme fragment having the ability to hydrolyze glycolipids and a substantially non-antigenic polymeric substance. In one preferred aspect of the invention, the conjugates include glucocerebrosidase or a substance having glucocerebrosidase activity covalently attached to an activated form of a polyalkylene oxide such as polyethylene glycol. In this regard, the polymer will

The present invention also provides methods of preparing the conjugates. The methods include reacting a substance having activity against glycolipids with a substantially non-antigenic polymeric substance under conditions sufficient to effect conjugation of the substituents while maintaining at least a portion of the anti-glycolipid activity. Such conditions include reacting the polymer with the enzyme-like substance in molar ratios ranging from about 5:1 to 125:1. The resultant conjugates have from about 1 to 25 polymeric strands attached to each enzyme-like substance.

The invention also provides methods of treating Gaucher's disease. In this aspect of the invention, treatment includes administering an effective amount of the conjugates described herein to patients or mammals requiring such therapy.

As a result of the present invention, conjugates having substantially prolonged enzyme-like activity against glycolipids <u>in vivo</u> are provided. The conjugates are substantially resistant to <u>in vivo</u> hydrolysis and thus uniquely allow less frequent administration of the therapeutic conjugate when compared to unmodified enzyme preparations and prolonged activity against accumulated glycolipids.

For a better understanding of the present invention, reference is made to the following description and its scope will be pointed out in the appended claims.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 is a graph demonstrating the comparative results of PEG-Glucocerebrosidase conjugates and unmodified glucocerebrosidase in vivo.

DETAILED DESCRIPTION OF THE INVENTION

The conjugates include substances having the ability to hydrolyze glycolipids. Such substances preferably have a glucocerebrosidase or glucocerebrosidase enzyme-like activity. These substances can be prepared or obtained from a variety of sources, including recombinant or mammalian extracted GCS. It is preferred that the enzyme included in the conjugate be prepared using recombinant techniques. In this regard, the recombinantly prepared glucocerebrosidase such as that disclosed in PCT WO 89/05850 may be used herein. The contents of this PCT publication are hereby incorporated by reference. Alternatively, glucocerebrosidase may be obtained from mammalian sources such as human placental tissue as disclosed in U.S. Patent No. 3,910,822. It is to be understood that other substances including pro-enzymes and fractions of enzymes or pro-enzymes can also be included in the conjugates of the present invention. As used herein, the expression "the ability to hydrolyze glycolipids" means any substance which demonstrates in vivo activity against mammalian glycolipids especially glucocerebroside. These substances are prepared by using techniques known to those of ordinary skill in the art such as tissue culture, extraction from plant or animal sources or by recombinant DNA methodologies. Transgenic sources of enzymes, pro-enzymes and fractions thereof are also contemplated. Such materials are obtained from

transgenic animals, i.e. mice, pigs, cows, etc. wherein the enzyme is expressed in milk, blood, or tissues. Catalytic antibodies specific for glycolipid catalysis are also contemplated. Such antibodies can be prepared using recombinant technologies where antibodies specific to a glycolipid binds to the glycolipid and cleaves the CHO-lipid bond. The method by which the enzymatic substance is prepared for the conjugates of the present invention is not limited to those described herein.

The substantially non-antigenic polymer substances included in the conjugates are preferably poly(alkylene oxides). Within this group of substances are alpha-substituted polyalkylene oxide derivatives such as methoxypolyethylene glycols or other suitable alkyl substituted derivatives such as C_1-C_4 alkyl groups. It is preferred, however, that the non-antigenic material be a monomethyl-substituted PEG homopolymer. Alternative polymers such as other polyethylene glycol homopolymers, polypropylene glycol homopolymers, other alkyl-polyethylene oxides, bis-polyethylene oxides and co-polymers or block co-polymers of poly(alkylene oxides) are also useful. In those aspects of the invention where PEG-based polymers are used, it is preferred that they have molecular weights of from about 1,000 to about 10,000. Molecular weights of about 2,000 to 7,500 are preferred and molecular weights of about 5,000 are especially preferred.

Alternative non-antigenic polymeric substances include materials such as dextran, polyvinyl pyrrolidones, polysaccharides, starches, polyvinyl alcohols, polyacryl amides or other similar non-immunogenic polymers. Those of ordinary skill in the art

will realize that the foregoing is merely illustrative and not intended to restrict the type of non-antigenic polymeric substances suitable for use herein.

As stated above, covalent modification of the enzyme-like material is preferred to provide the hydrolysis-resistant conjugate. The covalent modification reaction includes reacting a substance having the desired activity against glycolipids with a substantially non-antigenic polymeric substance under conditions sufficient to effect conjugations while maintaining at least a portion of the hydrolytic activity against glycolipids.

Oftentimes, the polymers are activated in order to effect the desired linkage with the enzymatically-acting substance. By activation, it is understood by those of ordinary skill in the art that the polymer is functionalized to include a desired reactive group. See, for example U.S. Patent Nos. 4,179,337 and 5,122,614 which are incorporated by reference herein. In these disclosures, the hydroxyl-end groups of polyalkylene glycols are converted and activated into reactive functional groups. One particularly preferred activated form of PEG for use in the present invention is poly(ethylene glycol)-N-succinimide carbonate. This activated polymer forms stable, hydrolysis-resistant carbamate (urethane) linkages with amino groups of the enzymatically active materials. Isocyanante-activated PEG's are also of use. While the references incorporated herein describe epsilon amino group modifications of lysine, other conjugation methods are also contemplated. Carbohydrate and/or acid group or other amino acid modifications are also within the scope of the present

invention. Covalent linkage by any atom between the enzyme and polymer is possible. Moreover, non-covalent conjugation such as lipophilic or hydrophilic interactions are also contemplated.

The process of the present invention includes preparing or providing the activated polymer and thereafter reacting it with a substance having the desired enzymatic or hydrolytic activity, in this case, the ability to hydrolyze glycolipids. The reaction is carried out in a buffer such as 0.1M phosphate buffer at a pH of from about 6.0 to about 8.0. The conjugate substituents are reacted with an appropriate amount of the polymer, which is typically present in a several-fold molar excess over the enzymatic-like substance. The polymeric excess will range from about 5 to about 125 molar ratio excess and preferably from about 50 to about 120 molar excess of the polymer to the enzyme-like substance. The reaction is carried out at temperatures of from about 0 to 25° C over time periods ranging from a few minutes to as long as 12 hours. Temperatures of from about 20 to about 25° C are preferred and time periods of around 1 hour are sufficient to carry out the conjugation reaction.

Following the conjugation reaction, the desired product is recovered using known techniques and purified using column chromatography or similar apparatus if necessary. Depending upon the reaction conditions, the conjugates have from about 1 to about 25 polymeric strands attached to the enzyme-like substance. By controlling the molar excess of the polymer reacted with the enzyme, for example, the artisan can tailor the number of polymeric strands attached. Conjugates

containing from about 5 to about 20 polymeric strands are preferred while conjugates containing from about 10 to 18 polymeric strands are most preferred.

Another aspect of the present invention provides methods of treatment for Gaucher's disease. The method includes administering an effective amount of the compositions described herein to alleviate the Gaucher's disease symptoms. Those of ordinary skill in the art will realize that the amount of the conjugate used in the method of the present invention will vary somewhat from patient to patient, however, conjugates capable of delivering from about 0.1 IU/kg to about 200^{+} IU/kg per administration and preferably 25 IU/kg are contemplated. The optimal dosing of the conjugate can be determined from clinical experience.

Further in this regard, the amount of the conjugate administered to treat Gaucher's disease is an amount that is sufficient to significantly reduce pathological glycolipid concentrations in vivo, and, in particular, the glycolipid glucocerebroside. The maximal dosage for humans is the highest dosage that does not cause clinically important side effects.

An important feature however is that by covalently combining the polymeric substance and the glycolipid hydrolyzing substance as described herein, the conjugates are substantially resistant to hydrolysis in vivo. The conjugates thus act on accumulated glucocerebroside in vivo to a greater extent than prior art compositions.

EXAMPLES

The following examples serve to provide further appreciation of the invention but are not meant in any way to restrict the effect of the scope of the invention.

EXAMPLE I

Modification of Glucocerebrosidase with SC-PEG

In this example, recombinant glucocerebrosidase was conjugated with the activated poly(ethylene glycol)-N-succinimide carbonate (SC-PEG) described in U.S. Patent number 5,122,614. The polymer had a molecular weight of about 5000. The recombinant glucocerebrosidase (rGCS) was prepared in accordance with the method described in PCT Publication No. WO 89/05850.

Procedures:

- 1.8 mg of recombinant glucocerebrosidase (rGCS) in 92 Mm NaOAc pH 5.8/10 % glycerol/18 % EtOH was dialyzed with 0.1 M Sodium phosphate buffer solution, pH 7.0 using a Centricon-10 (a product of the Amicon Corporation of Beverly, MA). The final concentration of the rGCS was ~0.5 mg/ml. 18 mg of SC-PEG (10-fold excess by weight = 120 molar excess) was added to the enzyme solution and the reaction mixture was stirred for 1 hr. at room temperature. The reaction was quenched by adding 0.1 M glycine. The unreacted PEG was removed by dialysis into a buffer solution having a pH of 6.5. The modification was checked by SDS-gel and the enzyme activity was measured

by Fluorimeter F-2000 Fluorescence Spectrophotometer, a product of Hitachi, Japan.

Results: The molecular weight of PEG-GCS was approximately estimated to be ~120,000-160,000 by SDS-gel. This corresponds to conjugates having about ~12-20 PEG strands attached. The enzyme activity was measured by using the artificial substrate 4-methyl- umbelliferyl-β-D-glucopyranoside (4-MUG) and 90 ± 5 % of the GCS activity was retained.

EXAMPLE II

Modification of GCS with SC-PEG

In this example, the procedure of example I is repeated using that a 20 fold molar excess of SC-PEG and same rGCS.

Procedures: 100mg of rGCS is dialyzed with 0.1M phosphate buffer, pH 7.0 using a Minisette (available from Filtron of Northborough, MA) to yield a 1 mg/ml solution. To this solution 167 mg of SC-PEG (a 20 fold molar excess) is added and stirred for 1 hr. at room temperature (~20° C). The reaction stopped by adding a 20 fold molar excess of 0.1M glycine. The unreacted reagents are removed by dialysis using the Minisette into a 20 millimolar NaOAC/phosphate 0.025% Tween 80 buffer, pH 6.5.

Results: PEG-GCS is estimated to have a molecular weight range of about 65,000-95,000 Daltons by SDS-PAGE. This suggests that between 1 and 7 PEG 5000 strands are attached to each enzyme molecule. It is estimated that greater than 80% activity of the GCS is retained.

As can be seen from the foregoing example, by varying the amounts of the reactants, the amount of PEG attached to the GCS can be varied. This, in turn, will vary the circulating life of the conjugate. Lower modifications yield conjugates with circulating lives substantially longer than native GCS but less than the higher modified PEG-rGCS made in Example I.

EXAMPLE III

In this example, the circulating half-lives of various glucocerebrosidase products was compared. The circulating half-life of both recombinant GCS and PEG-rGCS were determined in rats. Six rats about 300 ± 25 gm were used for this experiment. Three rats were injected i.p. with rGCS and three were similarly injected with PEG-rGCS at a dose of 60 IU/kg. At various time points the rats were bled and plasma prepared. The plasma was stored at 4°C until assayed. Glucocerebrosidase activity was determined using the 4-MUG fluorescent assay. The half-lives ($T_{½}$) are reported in the following table. The half-life for placental GCS was reported by Brady, et al, New England Journal of Medicine 291: 990-993 (1974); the half-life of Ceredase ™ was reported by Whittington, et al, Drugs 44(1) 72-93 (1992). The results are reported below.

CIRCULATING HALF-LIVES OF GLUCOCEREBROSIDASE

Preparation	T1/2
Placental GCS	25 minutes
Chemically Modified Placental GCS (Ceridase™)	3-11 minutes
Recombinant GCS[1]	approx. 1 hr (alpha phase)
PEG-rGlucocerebrosidase[2]	6-7 hours (alpha phase)

Ceridase™: a product of the Genzyme Corporation

[1] Prepared in accordance with the method of Ginns, et al in PCT WO 89/05850

[2] As prepared in Example I

As can be seen from the table, PEG-GCS conjugates prepared in accordance with the present invention have a substantially prolonged increase in circulating life when compared to unmodified enzymes.

EXAMPLE IV

In this example, the activity of the PEG-rGCS prepared in accordance with example I was compared to that of unconjugated rGCS prepared according to the method of Ginns, et al., supra, in a 72 hour plasma circulating half-life study. As was the case in example III, 3 rats were injected i.p. with 60 IU/kg with PEG-rGCS and 3 rats were injected with 60 IU/kg of rGCS.

Referring now to FIG. 1, it can be seen that not only does the conjugate provide the rapid activity associated with unmodified glucocerebrosidase at the outset, but the inventive conjugates provide higher levels of activity throughout a three day period. While applicants are not bound by theory, it is believed that such higher levels of enzyme activity in vivo over time serve to provide persistent activity against glucocerebroside. Such activity represents a major advance toward reducing residual glycolipid concentrations in Gaucher's Disease patients. This in turn will help alleviate some of the sequelae associated with later stage Gaucher's Disease such as enlarged spleen, liver and skeletal abnormalities.

While there have been described what are presently believed to be the preferred embodiments of the present invention, those skilled in the art will realize that changes and modifications may be made thereto without departing from the spirit of the invention. It is intended to claim all such changes and modifications as fall within the true scope of the invention.

WHAT IS CLAIMED IS:

1. A biologically active conjugate comprising a substance having the ability to hydrolyze glycolipids and a substantially non-antigenic polymeric substance.

2. The conjugate of claim 1, wherein said glycolipid is glucocerebroside.

3. The conjugate of claim 1, wherein said polymer is a poly(alkylene oxide).

4. The conjugate of claim 3, wherein said poly(alkylene oxide) is an alpha-substituted polyalkylene oxide derivative.

5. The conjugate of claim 3, wherein said poly (alkylene oxide) is selected from the group consisting of polyethylene glycol homopolymers, polypropylene glycol homopolymers, alkyl-capped polyethylene oxides, bis-polyethylene oxides and copolymers or block copolymers of poly(alkylene oxides).

6. The conjugate of claim 5, wherein said polymer has a molecular weight of from about 1,000 to about 10,000.

7. The conjugate of claim 6, wherein said polymer has a molecular weight of from about 2,000 to about 7,500.

8. The conjugate of claim 7, wherein said polymer has a molecular weight of about 5,000.

9. The conjugate of claim 1, wherein said substance is covalently linked to said polymer.

10. The conjugate of claim 1, wherein said substance is linked to said polymer via a carbamate linkage.

11. The conjugate of claim 1, wherein said polymeric substance is selected from the group consisting of dextran, polyvinyl pyrrolidones and other non-

immunogenic polymers.

12. The conjugate of claim 1, wherein said substance is glucocerebrosidase.

13. The conjugate of claim 12, wherein said glucocerebrosidase is of recombinant origin.

14. The conjugate of claim 12, wherein said glucocerebrosidase is of mammalian origin.

15. The conjugate of claim 14, wherein said mammalian
glucocerebrosidase is placental glucocerebrosidase.

16. The conjugate of claim 12, wherein said glucocerebrosidase is of transgenic origin.

17. The conjugate of claim 1, wherein said substance is selected from the group consisting of enzyme fragments, proenzymes and catalytic antibodies.

18. The conjugate of claim 1, wherein said conjugate comprises from about 1 to about 25 polymeric strands attached to said substance.

19. The conjugate of claim 18, wherein said conjugate comprise from about 5 to about 20 polymeric strands attached to said substance.

20. The conjugate of claim 19, wherein said conjugate comprises from about 10 to about 18 polymeric strands attached to said substance.

21. A method of preparing a conjugate having the ability to hydrolyze glycolipids, comprising reacting a substance having activity against glycolipids with a substantially non-antigenic polymeric substance under conditions sufficient to effect conjugation of said substance and said polymeric substance while maintaining at least a portion of the substance's activity.

22. The method of claim 21, wherein said polymer is a poly (alkylene oxide).

23. The method of claim 22, wherein said polyalkylene oxide is a alpha-substituted polyalkylene oxide derivative.

24. The method of claim 22, wherein said poly (alkylene oxide) is a polyethylene glycol.

25. The method of claim 24, wherein said polyethylene glycol is a methoxypolyethylene glycol.

26. The method of claim 24, wherein said polyethylene glycol is activated to include a linking group for attaching said polymer to said substance.

27. The method of claim 26, wherein said activated polyethylene glycol is PEG-N-succinimidyl carbonate.

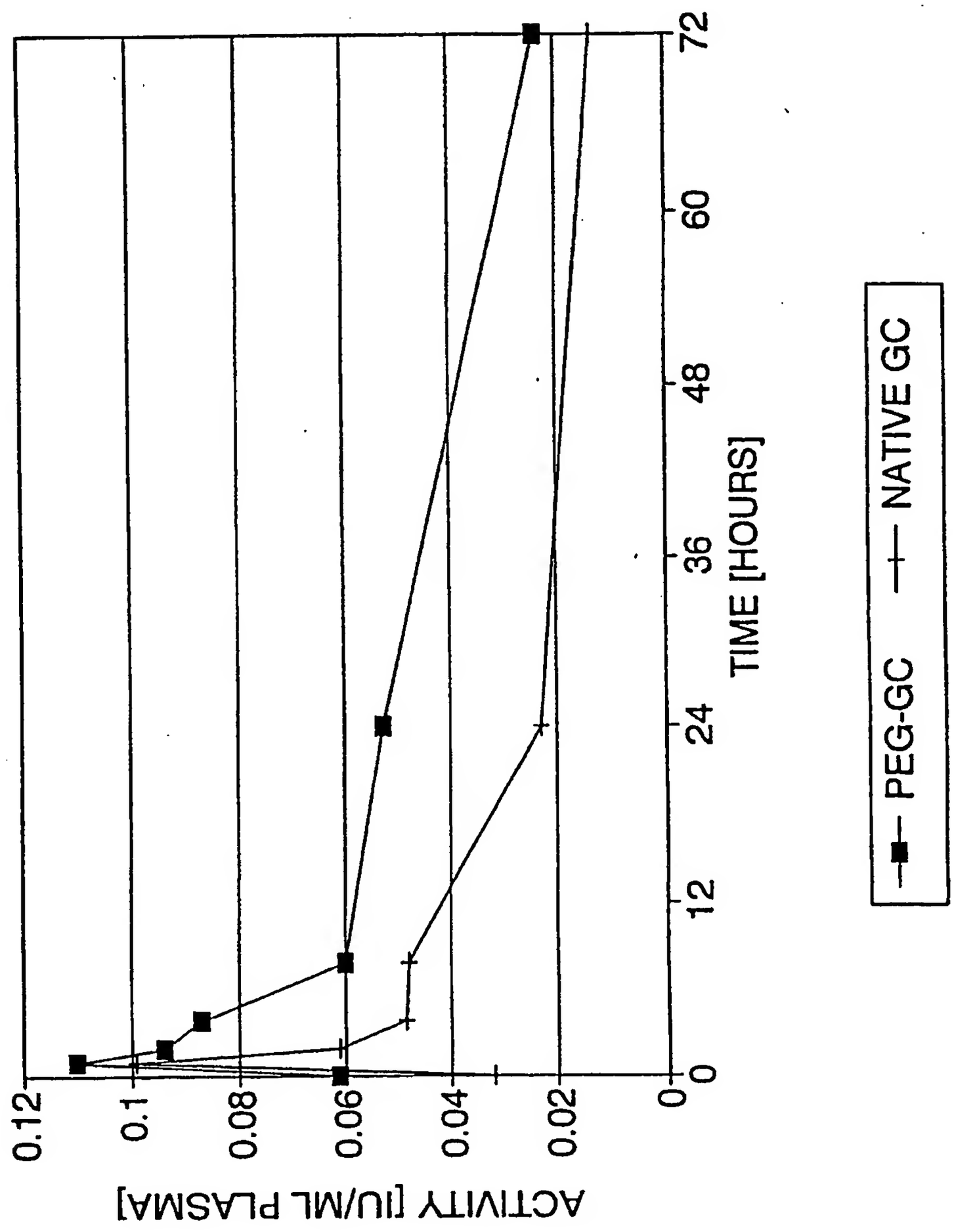
28. The method of claim 26, wherein said activated polyethylene glycol is PEG-isocyanate.

29. The method of claim 21, wherein said conditions include reacting said polymer with said substance in a molar ratio of from about 5:1 to 125:1.

30. The method of claim 29, wherein said condition include reacting said polymer with said substance in a molar ratio of from about 50:1 to 120:1.

31. A method of treating Gaucher's disease comprising administering an effective amount of the composition of claim 1.

FIGURE 1


ACTIVITY [IU/ML PLASMA]
0.12
0.1
0.08
0.06
0.04
0.02
0
TIME [HOURS]
0
12
24
36
48
60
72
PEG-GC
NATIVE GC

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/11920

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/00, 47/48, 37/54; C12N 9/42, 9/96, 11/00
US CL :424/94.3, 94.61; 435/174, 188, 209

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/94.3, 94.61; 435/174, 188, 209

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,179,337 (Davis et al.) 18 December 1979, entire document.	1-9, 11-26, 28-31
Y	US, A, 5,122,614 (Zalipsky) 16 June 1992, entire document.	1-31
Y	JOURNAL OF LABORATORY AND CLINICAL MEDICINE, Volume 96, No. 4, issued October 1980, J.D. Humphreys et al. "Enhanced Stability of Erythrocyte-Entrapped Glucocerebrosidase Activity", pages 682-692, particularly pages 682-685 and 689-691.	1-31
Y	US, A, 3,910,822, (Pentchev et al.) 07 October 1975, entire document.	14, 15, 31

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
04 February 1994	FEB 14 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer ROBERT WAX Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/11920

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DRUGS, Volume 44, No. 1, issued January 1992, R. Whittington et al. "Aglucerase, A Review of Its Therapeutic Use in Gaucher's Disease", pages 72-93, particularly pages 72-75 and 79-88.	14,15,31
Y	WO, A, 90/07573 (Rasmussen et al.) 12 July 1990, entire document.	13-16, 31
A	JOURNAL OF CONTROLLED RELEASE, Volume 11, issued January 1990, F. Fuertges et al., "The Clinical Efficacy of Poly(Ethylene Glycol)-Modified Proteins", pages 139-148, entire document.	1-31

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/11920

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CAS, BIOSIS, EMBASE, MEDLINE, LIFESCI
search terms: glucocerebrosidase, half life or clearance or stability or lifetime, conjugat? or crosslink? or link?, polyalkylene or polypropylene or polyethylene or polyvinyl or dextran or methoxypolyethylene, protein# or enzyme# or polypeptide#